IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Rima Kaddurah-Daouk *et al.*

Application No.: 10/718,765 | Confirmation No.: 1461

Filed: November 21, 2003 | Art Unit: 1639

For: USE OF CREATINE OR CREATINE ANALOGS FOR THE TREATMENT OF DISEASES OF THE NERVOUS SYSTEM | Examiner: J. Lundgren

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF BELINDA TSAO NIVAGGIOLI, Ph.D.
UNDER 37 CFR §1.132

Dear Sir:

I, Belinda Tsao Nivaggioli, Ph.D., a citizen of Canada, residing in Atherton, California, hereby declare as follows:

1. I am presently the Chief Executive Officer of the Avicena Group, Inc. (Palo Alto, California). I have been working in the area of pharmaceuticals and nutraceuticals for approximately 14 years. A copy of my curriculum vitae is attached as Appendix A.

2. I have read the above-referenced application and presently pending claims 22-40 (included herewith as Appendix B). It is my understanding that the invention is directed to methods of treating Huntington's disease by administering a therapeutically effective amount of creatine, creatine phosphate, or a salt thereof.

3. In addition, I understand that claims 22-29 are rejected under 35 U.S.C. § 103 (a), first paragraph, as being unpatentable over Ericinska *et al., Journal of Cerebral Blood Flow and Metabolism*, 9:2-19 (1989); in view of Beal *et al., Journal of Neurochemistry* 57(3):1068-1073 (1991), in view of Roberts *et al., American Journal of Physiology* 243(6):H911-H916 (1982). In particular, the Examiner states the Ericinska *et*

al. to teach that "a major pathological component of CNS complications is ATP depletion." Furthermore, according to the Examiner, Beal *et al.* shows that "when AOAA is introduced to straital tissue that ATP levels were depleted and resulted in striatal lesions that closely resemble[d] Huntington's disease." In addition, the Examiner relies on Roberts to teach that "the feeding of creatine analogs delays ATP depletion and the onset of rigor in ischemic heart tissue."

4. It is my opinion that the treatment of Huntington's disease using creatine, as claimed in the present application, would not have been obvious over the cited references. As described below, the administration of creatine to Huntington's disease patients is effective to slow progress of the disease. This is surprising because at the time the application was filed, no cure, effective therapy, or way to slow down progression of Huntington's disease was known (Leegwater-Kim *et al.*, NeuroRx. 2004 January; 1(1): 128–138).

5. Creatine has been shown to slow the progression of Huntington's disease when 10 g of creatine were administered to subjects daily for a period of twenty four months. As described below, an open-label pilot study in gene positive preclinical and affected patients with Huntington's disease was done to assess the tolerability, safety, and efficacy of high-dose creatine supplementation. Serial ^{31}P MR spectroscopy (MRS) of muscle was used to assess defects and monitor changes in energy metabolism. ^{1}H-MRS was used to assess brain creatine concentrations.

Thirteen genetically confirmed patients with Huntington's disease were recruited. Three were clinically unaffected and 10 were affected (clinical stages 1 to 3). Four age-matched normal spouse controls were recruited. Exclusion criteria were any intercurrent medical condition or drug or alcohol abuse. The subjects took 10 g per day of creatine and were advised to avoid caffeine and dehydration. Biochemical and hematologic tests were performed at baseline and 3 monthly intervals. Patients were assessed clinically using the United Huntington's Disease Rating Scale [UHDRS] and by MR spectroscopy [MRS] at baseline and 6 and 12 months.

All subjects and controls tolerated creatine treatment, apart from mild nausea and diarrhea. In two subjects, the daily dose was reduced to 5 g after 6 months owing to diarrhea, which settled. One subject discontinued treatment at 6 months and two more discontinued treatment after 12 months owing to poor compliance. Another subject was

removed from the study after 12 months because of a rise in serum creatinine (231 mmol/L; normal range 60 to 120).

TMS, functional capacity, and neuropsychology testing showed no significant difference at 12 months. There was no deterioration, and these data indicate stabilization of Huntington's disease symptoms and a delay in the progression of the disease.

After 24 months of creatine treatment, there was no significant change in the mean TMS, functional capacity scores, or neuropyschological testing. MRS studies demonstrated that creatine was elevated in vivo in both brain and in muscle as assessed by N-acetyl aspartate (NAA)/ creatine and phosphocreatine (PCr)/ATP ratios. Mean body weight was slightly increased (72.1 ± 15.6 kg) as compared with baseline (71.0 ± 12.8 kg).

This study showed that 10 g per day of creatine for 24 months is safe and well tolerated. Brain proton spectroscopy demonstrated that creatine crosses the blood– brain barrier and results in increased cerebral concentrations. Muscle MRS, however, demonstrated only a transient increase in PCr:ATP or PCr:inorganic phosphate ratios at 6 months, but no change at 12 and 24 months.

TMS, functional capacity and neuropsychological testing using the UHDRS showed no significant difference at 2 years from baseline, although the trend was a decline in function, as expected with a cohort of subjects with Huntington's disease. There were differences between the subjects at similar clinical stages, with some showing improvement in the scores, suggesting creatine may be able to slow the progression or even treat Huntington's disease.

6. The affects of administering creatine to Huntington's disease subjects was also studies in a two-phase open-label study which was conducted to better determine an optimal dose of creatine. A dose-escalation study (10-40 grams per day) was conducted to determine the maximally tolerated dose (MTD) followed by a de-escalation phase to assess whether brain and serum levels of creatine might be maximal at doses lower than the MTD. Ten subjects were enrolled and followed prospectively for two weeks at each done level increasing in 5-gram increments during dose escalation that lasted 13 weeks. Assessments at each visit included UHDRS, EKG, vital signs, clinical safety and research labs. MRI spectroscopy was conducted prior to baseline at peak done (40 grams) and one month after de-escalation to either 30 or 15 grams daily. To determine an optimal dose, pharmacokinetic as well as clinical data were considered. Once the maximal dose was reached, subjects were assigned one of two lower doses previously taking (15 grams a

day (n = 5) or 30 grams a day (n = 5)). Serum creatine was assessed at baseline, at the end of each 2-week dose escalation step and at the end of the de-escalation phase to assess the correspondence between serum and brain levels of creatine at steady state.

All subjects tolerated up to 40 grams/day of creatine during the does escalation and there were no withdrawals. However, there were increased numbers of adverse events at 35 and 40 grams daily, chiefly low-grade nausea and diarrhea. There were no serious adverse events, no significant laboratory abnormalities and no EKG alterations at any of the doses. Although individual subject's creatinine and BUN levels remained within normal clinical ranges, there were changes in average levels. Average creatinine levels increased in a dose-dependent manner from baseline (1.0 ± 0.2 mg/dl) to 30 grams a day (1.28 ± 0.16 mg/dl). Creatinine levels remained stable up to 40 grams/day but decreased in the de-escalation phase. BUN levels did not increase with higher doses during dose escalation and actually decreased somewhat at 15, 25, 30 and 40 grams daily creatine doses perhaps because of an emphasis placed on good hydration. Serum creatine levels increased with each dosage step until they reached a plateau at 30-35 grams/day and actually declined at 40 grams daily. Changes in UHDRS subscores were not significant. Some subjects felt worse, however, on 35 and 40 grams daily, which could have been due to GI side effects. Brain levels of creatine in the frontal cortex, as assessed by MRI spectroscopy indicated continued increases in brain creatine throughout the dosage range indicating that these high doses are not saturating brain levels. There was a dose dependent suppression of 8OH2'dG to levels greater than seen when subjects were administered 8 grams of creatine per day. Higher doses were associated with further suppression and doses greater than about 25 grams daily were associated with maximum suppression of serum 8OH2'dG levels similar to normal controls. These data suggested that doses higher than 10 grams daily are safe, tolerable and have greater peripheral and brain bioavailability, and are associated with further suppression of a disease related mechanism (oxidative stress as measured by 8OH2'dG). Based on all of this data, 30 grams daily provided excellent tolerability, high bioavailability and maximal biomarker suppression.

Subjects at the end of this study were given the option to continue a long-term study to evaluate the long-term safety and tolerability of high dosage of creatine. Subjects have been followed for nine months on creatine. There have been no significant clinical changes (UHDRS subscores) up to 9 months and there have been no serious adverse effects. Adverse events have been infrequent and mild to moderate in grade and 30 grams daily creatine has been well-tolerated for 9 months. Laboratory abnormalities

have been mild. One subject had a Grade 2 (moderate) SGPT level after one-month on 30 grams/day, but his normalized by month 3 without dose adjustment.

Morphometric neuroimaging was performed in all subjects. Longitudinal data from up to three years prior to initiating creatine was available on 6 or the 10 subjects. The rate of thinning of cortical regions in Huntington's disease was modeled based on the longitudinal data and a change in rate was determined for each region of the group while on creatine. Creatine reduced the rate of thinning in almost every region, and the rate of change was determined for each region for the group while on creatine. Creatine reduced the rate of thinning in almost every region, and the rate of change was statistically significant for several regions and amounted to about a 30% slowing.

7. It has been found that administering creatine to subjects suffering from Huntington's disease stabilizes the subjects' total motor scores and functional capacity scores, while reducing the rate of cortical thinning. This is surprising because at the time the application was filed, no cure, effective therapy, or way to slow down progression of Huntington's disease was known (Leegwater-Kim *et al.*, NeuroRx. 2004 January; 1(1): 128–138). For at least the above reasons, it is my opinion that creatine has surprising and unexpected therapeutic activity for the treatment of Huntington's disease.

8. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title XVIII of the United States Code, and that such willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

March 17, 2008

Belinda Tsao Nivaggioli, Ph.D. Date

APPENDIX B
Pending Claims
U.S.S.N. 10/718,765

22. A method for treating a subject with Huntington's disease, comprising administering to the subject an effective amount of creatine, creatine phosphate or a salt thereof sufficient to reduce or ameliorate Huntington's disease, and further comprising coadministering to the subject a neurotransmitter, a neurotransmitter analog, an immunomodulating agent, or an immune suppressive agent.

23. A method for treating a subject with Huntington's disease, comprising administering to the subject an effective amount of creatine, creatine phosphate or a salt thereof sufficient to reduce or ameliorate Huntington's disease, and further comprising coadministering to the subject a a steroid.

24. The method of claim 22, wherein said subject is administered an effective amount of creatine.

25. The method of claim 22 wherein the subject is a mammal.

26. The method of claim 25, wherein the subject is human.

27. The method of claim 23, wherein said subject is administered an effective amount of creatine.

28. The method of claim 23, wherein the subject is a mammal.

29. The method of claim 28, wherein the subject is human.

30. A method for reducing progression of Huntington's disease in a subject, comprising administering to the subject an effective amount of creatine or a salt thereof sufficient to reduce progression of Huntington's disease in said subject.

31. The method of claim 30, wherein the subject is a mammal.

32. The method of claim 31, wherein the subject is human.

33. A method for reducing progression of Huntington's disease in a subject, comprising administering to the subject an effective amount of creatine phosphate or salt thereof sufficient to reduce progression of Huntington's disease in said subject.

34. The method of claim 33, wherein the subject is a mammal.

35. The method of claim 34, wherein the subject is human.

36. A method for treating a subject with Huntington's disease, comprising administering to the subject an effective amount of creatine, creatine phosphate or a salt thereof sufficient to treat Huntington's disease, such that said subject is treated for Huntington's disease.

37. The method of claim 36, wherein said subject is administered an effective amount of creatine.

38. The method of claim 36, wherein the subject is a mammal.

39. The method of claim 38, wherein the subject is human.

Appendix A

Belinda Tsao Nivaggioli, Ph.D.
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WORK EXPERIENCE

The Avicena Group, Inc., Palo Alto, CA

Chief Executive Officer	1/05 - Present
Chief Operating Officer	12/01 – 1/05
Vice President of Operations	12/00 – 12/01
Director of Product Development	9/99 – 11/00

Avicena Group, Inc. (OTCBB: AVGO) is a late stage biotechnology company focused on developing products based on its proprietary understanding of the regulation of cellular energy processes. The company's core technologies, supported by a robust IP portfolio, have broad applications in both pharmaceuticals and dermaceuticals. Avicena's pharmaceutical program centers on rare neurological disorders (orphan diseases). The company is currently analyzing data from its Phase IIb/III trial in ALS (Amyotrophic Lateral Sclerosis, or Lou Gehrig's disease). Near term, Avicena intends to initiate a Phase III trial in Huntington's disease and a Phase III trial in Parkinson's disease. Avicena's science is well established and its products are safe and well tolerated. Unlike traditional biotechnology companies, Avicena's clinical programs are largely funded by government and non-profit organizations. Avicena presently derives revenue from the sale of proprietary ingredients to skin care manufacturers.

Oral-B Laboratories, A Gillette Company, Belmont, CA

Manager, Product Development, Floss and Interdental	11/98 – 9/99
Group Leader, Floss and Interdental	11/95 – 11/98

Development, scale up and manufacture of new interdental products, such as flosses and interdental devices. Involved in project management, procurement and setup of manufacturing line in the factory, market research, clinical studies, launch planning, preparation of sales materials and manufacturing logistics. Supervision of engineers and technicians.

The Gillette Company, Boston, MA
Research Scientist, Corporate Research and Development 11/94 - 11/95
Synthesis and microbiological assays of novel antimicrobial agents for treating plaque and gingivitis. Synthesis and studies of acidochromic materials. Worked closely with various business units to develop strategic business plans. Supervision of technicians and students.

Massachusetts Institute of Technology, Cambridge, MA 1993 - 1994
Postdoctoral Associate in Prof. Julius Rebek Jr.'s group. Studied chemical nucleases, self-replicating systems and combinatorial libraries. Collaborated with Prof. Alan Hatton, in the Department of Chemical Engineering in the study of water-soluble polymers using NMR and fluorescence spectroscopy.

EDUCATION

University of Toronto, Toronto, ON, Canada.
Ph.D. in Bioorganic Chemistry (January 1993). Study of conformation catalysis of decarboxylation by host-guest chemistry. University of Toronto Open Fellowship (1990 - 1992).

M.Sc. in Bioorganic/Physical Organic Chemistry (May 1990). University of Toronto Open Fellowship (1988 - 1990).

Oberlin College, Oberlin, OH, USA
A.B. (Hons) in Organic Chemistry (1988). Li Shu Fan Foundation Scholarship (1984 - 1988).

ASSOCIATIONS

Member of American Chemical Society (ACS), American Association for the Advancement of Science, the Society of Plastics Engineers, International Association of Dental Research

PUBLICATIONS / REFERENCES

Available upon request. As of March 1998, 5 papers were published in international journals, 6 presented at international conferences, 1 patent granted and 2 patent applications are pending.

LANGUAGES

English, Chinese and French (proficient in reading, writing and conversation).

HONORED MEMBERS

Medicine's Who's Who 2004